

Semi-Continuous Production Process of Corn Vinegar in Stirred Tank Reactor Using Fixation of *Acetobacter aceti* WK on Surface of Loofa Sponge

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ABSTRACT

Previous study by the authors showed that an alternative process to an airlift fermenter in the green vinegar production process could use a loofa sponge (a natural agricultural fibrous material) as the supporting material for *Acetobacter aceti* WK. Subsequently, aim of this study was production of green vinegar in a stirred tank reactor (STR). The filtered corn wine was supplied to the STR using fixation of *A. aceti* WK cells on the surface of a loofa sponge cut to a thickness of 2.5 cm. The loofa sponge was left in the STR until 60% of fermenting volume had been used. The charging rate of fresh, filtered corn wine for a semi-continuous production process was investigated. The highest acidification rate (ETA) was achieved at a charging rate of 30%. After 14 d of the adaptation period, the average acidity of $4.1 \pm 0.1\%$ was produced during six cycles of fermentation at 30°C and 1vvm aeration supply within 40 d, while the ETA was in the range 0.0028%/h to 0.0067%/h. Scanning electron microscopy (SEM) showed that the surface of the loofa sponge acted as a good fibrous structure for cell fixation of *A. aceti* WK in the STR.

Keywords: semi-continuous production process, corn vinegar, stirred tank reactor, *Acetobacter aceti* WK, loofa sponge

INTRODUCTION

During past decades, various vinegar production processes using different reactors have been developed. The continuous stirred tank reactor (Aeschbach, 1982; Nodes, 1986), packed bed bioreactor (Horiuchi *et al.*, 2000) and airlift fermenter (Krusong *et al.*, 2005) are examples of applications. Other processes, including high cell density (Tamai *et al.*, 1997), fermentation control (Visessanguan, 1988), the semi-continuous

process (Krusong *et al.*, 2002) and the trickle process (Fleury, 1995) have also been investigated. However, the fixation of *Acetobacter aceti*, using supporting material, was developed and promoted as an alternative “quick process” for vinegar production. Several different supporting materials were recommended, such as lipophilic fibers (Okuhara, 1987), charcoal pellets derived from waste mushroom medium (Horiuchi *et al.*, 2000) and loofa sponge (Krusong *et al.*, 2007).

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Loofa (*Luffa cylindrica*) is one of the vegetable sponges and belongs to a group of gourds (Stephens, 2007). The dried fibrous part of a loofa is normally used as a washing sponge or strainer (www.thefreedictionary.com), scrubbing pads, bath mats and other items (www.loofa.info).

A previous study (Krusong *et al.*, 2007) found that loofa was recommended as an excellent supporting medium for *A. aceti* WK for semi-continuous fermentation of vinegar production in an airlift fermenter. It was considered that loofa could help promote the new process as a “green” vinegar production process. Loofa is a natural agricultural fibrous material that can decompose naturally, in contrast to plastic fibrous material. Additionally, loofa has been fully approved with regard to any food safety concerns because it is an edible vegetable.

In this study, therefore, loofa sponge, with its natural fibers, was further investigated to establish its suitability as a supporting medium for use with *A. aceti* WK in the STR. The main focus of the current study was to develop a semi-continuous vinegar production process from corn wine as an alternative vinegar production process for small and medium enterprises.

MATERIALS AND METHODS

Loofa sponge preparation

Dried fibrous loofa sponge was organically planted and harvested for use as supporting material with *A. aceti* WK. It was cut into pieces 2.5 cm thick as mentioned in previous investigations (Krusong *et al.*, 2007) and shown in Figure 1. After cutting, each piece of dried loofa sponge was washed under running tap water and dipped in vinegar containing 4% acetic acid for sterilization (Krusong, 2008).

Yeast strain and corn wine fermentation

Saccharomyces cerevisiae M30, a



Figure 1 Characteristics of loofa sponge after cutting for use as supporting material for the fixation of *A. aceti* WK on its surface during corn vinegar fermentation in a stirred tank reactor (STR).

flocculate yeast, which was provided by the Yeast Biotechnology Laboratory, Department of Microbiology, Kasetsart University, Thailand, was used for corn wine fermentation. The medium used for corn wine fermentation had pH 5.5 and consisted of (per 16 L of water): corn, 0.8 kg; sucrose, 3.2 kg; $(\text{NH}_4)_2\text{SO}_4$, 8 g; and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.2 g. Fermentation was conducted using the cell recycle process (Figure 2a). The rate of cell recycling was 50 mL/min, as recommended by Krusong *et al.* (2002). Fermentation was controlled at 32°C for 48 h.

Bacterial strain and vinegar fermentation in stirred tank reactor (STR)

A. aceti WK, a strain producing acetic acid, was screened and adapted for vinegar production using corn wine as a substrate. The complex medium used for vinegar production consisted of (per 1 L of water): glucose, 5.0 g; yeast extract, 2.5 g; and peptone, 1.0 g. The total acidity concentration of the vinegar and alcohol was controlled, with the concentration at start-up being 7% that consisted of 3.5%(v/v) vinegar and

3.5%(w/v) alcohol. The amounts were prepared and added to the medium in a 50-liter STR. The vinegar was fermented with aeration at 1 vvm. The cycles of the semi-continuous fermentation process were studied, as adapted from Krusong *et al.* (2002, 2007). Each cycle started when the alcohol content of the previous fermenting broth was diminished to 0.5-0.8%(w/v). At this stage, fresh corn wine with 7% total concentration (consisting of 1.0%(v/v) acidity of vinegar and 6%(w/v) alcohol) from the fresh corn wine tank was pumped into the 50-liter STR at a suitable charging rate, while the fermenting broth from the 50-liter STR was pumped into the finished vinegar tank. The charging rate of filtered fresh corn wine to the 50-liter STR was controlled to match the discharge rate of fermenting broth to the finished vinegar tank. The charging rate of fresh corn wine at 10, 20, 30 and 40% was studied to determine the maximum acidification rate. The experimental apparatus and schematic diagram are shown in Figures 2b and 3, respectively.

Sensory evaluation of corn wine and corn vinegar

The aromas of corn in the corn wine and corn vinegar were tested by sensory evaluation and compared with natural, steamed, fresh corn. A five-

point hedonic scale was used in this study. According to the procedure of Cochran (1963), nine trained panelists, who frequently tested natural steamed fresh corn, participated in this study.

Analytical procedure

The invert sugar (as measured by the by Lane and Eynon method) and acidity (expressed

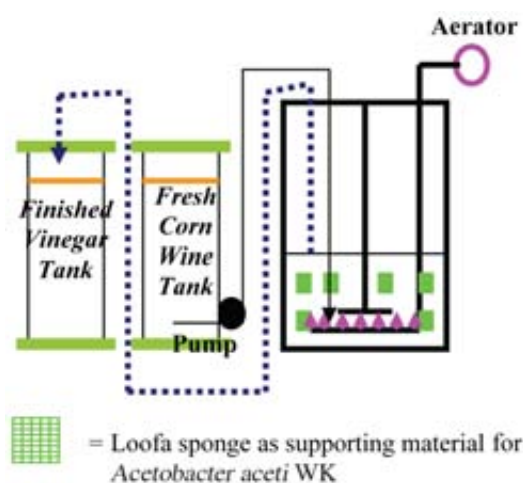


Figure 3 Schematic diagram of experimental apparatus for the corn vinegar production process, using dried loofa sponge as the supporting material for *A. aceti* WK in a 50-liter STR.



Figure 2 Experimental apparatus: (A) corn wine fermentation using a cell recycle system at 50 mL/min; and (B) corn vinegar fermentation in a 50-liter STR.

in term of acetic acid by titration) were analyzed according to AOAC (1995). Alcohol content was analyzed by an Ebulliometer and compared with an alcohol hydrometer, while pH was measured with a pH meter, model JENWAY 3510, UK. The microstructure of *A. aceti* WK on the surface of the loofa sponge in the fermenting broth was examined using a scanning electron microscope (SEM). Samples were vacuum dried and then sputtered with gold and photographed. Each image was taken on a JEOL JSM-5410LV (JEOL, Tokyo) scanning electron microscope. The dissolved oxygen (DO) was measured by an Oxygen Amplifier Model 170 and reported in term of the percentage of air saturation. The acidification rate was defined as the increase in acetic acid concentration (AAc) during fermentation and expressed in terms of %/h.

Statistical analysis

All experiments were carried out in triplicate, using a completely randomized design (CRD). Data were subjected to analysis of variance and Duncan's new multiple range test (DMRT), using the SPSS 10.0 for Windows pocket program, to determine if there was a significant difference ($P \leq 0.05$) in the mean aroma values of corn wine.

RESULTS AND DISCUSSION

Corn wine was prepared and used as the substrate for vinegar production. The cell recycle system (Figure 2a) involving the flocculate yeast, *S. cerevisiae* M30, was used for wine production. During fermentation, *S. cerevisiae* M30, which had flocculated to the bottom, was pumped to the top of the fermentation vessel at 50 mL/min, which was the cell recycle rate recommended by Krusong *et al.* (2002). The more viable the yeast cells were, the greater the alcohol content increase in the wine. As shown in Figure 4, corn wine containing 8.4% alcohol content and 0.5% acidity was obtained after 48 h of fermentation at 32°C. The cell recycle

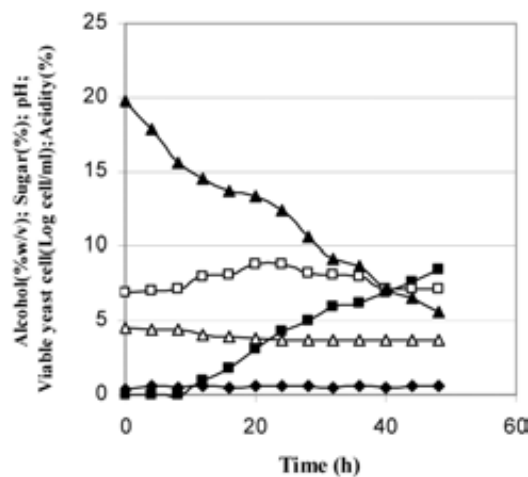


Figure 4 Corn wine fermentation by *S. cerevisiae* M30 in a 20-liter fermenter with a cell recycle process over 48 h at 32°C. Alcohol, ■; viable yeast cells, □; sugar, ▲; pH, △; acidity, ◆.

system of *S. cerevisiae* M30 promoted faster alcohol production than a non- cell recycle system containing 9% alcohol with 150 h of fermentation, as mentioned in Krusong *et al.* (2002). This may have been due to the dispersion of yeast cells in the fermenting broth after cell recycling, instead of the yeast cells settling to the bottom of the fermenting vessel as normally occurred. Additionally, the corn wine produced had a satisfactory aroma profile as judged by the trained panelists with experience in testing natural, steamed, fresh corn. The results in Table 1 reveal that there was no significant difference in the corn aroma between the wine and vinegar. However, the acceptability of the corn wine and corn vinegar were significantly different compared with natural steamed fresh corn, but the acceptable corn aroma in vinegar was noticeable.

The fixation of *A. aceti* on the surface of fibrous material was part of an alternative vinegar production process, called the “quick process”. Usually, most fibrous supporting material has been plastic, consisting of polypropylenes, polyethylenes, polystyrenes, polyethylene

Table 1 Mean values for sensory evaluation of corn wine and corn vinegar compared with natural, steamed, fresh corn (n = 9).

Product	Corn aroma score*	Acceptance* (%)
Natural steamed fresh corn	3.88 ^a	85.4 ^a
Corn wine	3.25 ^b	78.8 ^b
Corn vinegar	3.17 ^b	76.3 ^b

* Mean value followed by the same superscript in the same column indicates not significantly different by Duncan's new multiple range test ($P \leq 0.5$).

terephthalate or polyurethanes (Okuhara, 1987). For this study, dried loofa sponge (an organic fibrous material) was chosen instead of plastic material. It has been proved already that it could support cells of *A. aceti* WK on its surface (Krusong *et al.*, 2007). The current study aimed to prove that loofa sponge could be used for vinegar production in STR, as demonstrated in Figure 2b. After dipping in vinegar containing 4% acetic acid for sterilization, the 2.5-cm-thick, dried loofa sponge was submerged in the fermenting broth. The preliminary study showed that dried loofa sponge could be put into the STR to make 60% of the fermenting volume. The characteristics of the loofa sponge in the fermenting broth during corn vinegar production are shown in Figure 5.

A previous report (Krusong *et al.*, 2002)

**Figure 5** Loofa sponge used as supporting material for *A. aceti* WK in fermenting broth during corn vinegar production in a 50-liter STR at 30°C.

indicated that a 40% charging rate of fresh wine was recommended for the semi-continuous production process of corn vinegar in an airlift fermenter. However, the current study investigated varying the charging rate of corn wine between 10 and 40%, to determine the optimum rate for production in an STR, instead of an airlift fermenter. As shown in Table 2, *A. aceti* WK required 14 d for its adaptation when inoculated in the new fermenting medium containing 7% total concentration (3.5% acidity of vinegar plus 3.5% alcohol) for the range in the charging rate. There was a reduction in the alcohol content of 0.5-0.8% in the medium. However, the adaptation period for *A. aceti* WK cultivated in an STR was longer than the period mentioned by Krusong *et al.* (2007) using an airlift fermenter. Using DO measurements, there was more oxygen (75-85% air saturation) in the airlift fermenter compared to the STR (70-75% air saturation). The results in Table 2 show the effect of charging rate on the acidification rate (ETA) using *A. aceti* WK in an STR. The low acid content of vinegar was significant when a charging rate of 10-20% was used. This was due to the low alcohol content in the fermenting mash after charging the filtered fresh corn wine from the fresh corn wine tank to the 50-liter STR. Additionally, a significantly greater alcohol content in the fermenting mash was observed when the charging rate of fresh corn wine was 30%-40%. However, the highest ETA, which was significant, was obtained when the charging rate was 30%. This result indicated that a charging

Table 2 Effect of charging rate of fermenting medium on acidification rate of *A. aceti* WK in an STR, using loofa sponge as the supporting material at 30°C.

Charging rate (%v/v)	Adaptation period ¹ (d)	Acidification rate ² (%/h)	Average Acidification rate ³ (%/h)
10	14	0.0010-0.0018	0.0014 ^c
20	14	0.0014-0.0021	0.0018 ^c
30	14	0.0022-0.0039	0.0031 ^a
40	14	0.0019-0.0032	0.0026 ^b

¹ Adaptation period is the lag phase needed in the growth cycle of *A. aceti* WK to oxidize the initial alcohol in the fermenting mash to 0.5%.

² Acidification rate is the increase in acetic acid concentration.

³ Mean followed by the same superscript in the same column indicates not significantly different by Duncan's new multiple range test ($P \leq 0.5$).

rate of 30% provided an appropriate environment for the activity of *A. aceti* WK in the STR. The suitable alcohol concentration from fresh corn wine in the fermenting medium was oxidized to acetic acid under a DO value of 70-75% air saturation in the STR.

The investigation of semi-continuous vinegar production was repeated using a charging rate of 30% for the charged fresh corn wine into the fermenting mash after the first complete adaptation period of *A. aceti* WK. As shown in Figure 6, the results confirmed that *A. aceti* WK cells took 14 d for their adaptation in fresh corn wine consisting of 3.5% acidity of vinegar and 3.5% alcohol at the beginning of vinegar production in the STR. The slight increase in acidity that occurred while there was the gradual reduction in the alcohol content in fermenting mash was investigated. Theoretically, *A. aceti* WK had successfully adapted when the alcohol content in fermenting medium was reduced to 0.5-0.8%. Then, the filtered fresh corn wine with 7% total concentration (consisting of 1.0%(v/v) acidity of vinegar and 6%(w/v) alcohol) from fresh corn wine tank was charged into the fermenting mash in the 50-liter STR. The charging rate was controlled at 0.3125 L/h. Simultaneously, the fermenting mash containing acetic acid was discharged at the same rate charging rate. Within the next 40 d after the adaptation period, six cycles

of semi-continuous vinegar production were conducted. The first two cycles required a longer fermentation time, while subsequent cycles were shorter. This phenomenon was due to the adaptation of *A. aceti* WK. An average of $4.1 \pm 0.1\%$ acetic acid was obtained during these six cycles. The acidification rate (ETA) indicated by the hourly increase in the acid concentration was calculated, producing a range of 0.0028 to 0.0067%/h of ETA from those fermentation cycles. Additionally, the comparison of ETA by *A. aceti*

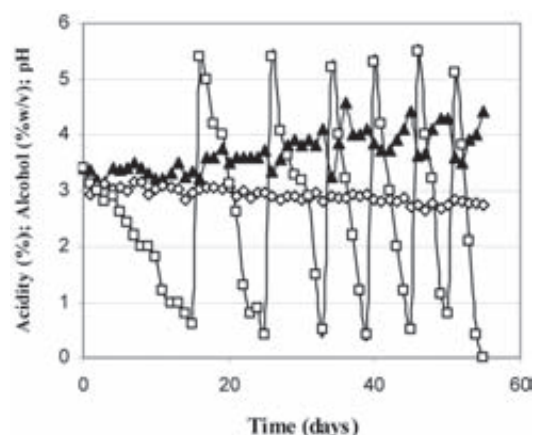


Figure 6 Semi-continuous production of corn vinegar by *A. aceti* WK in a 50-liter STR. Fermentation conditions were controlled at 30°C, 1.0 vvm aeration rate and 0.3125 L/h charging rate. Alcohol, \square ; acidity, \blacktriangle ; pH, \diamond .

WK in an airlift fermenter and in the STR of this study is shown in Table 3. The ETA in the STR was significantly lower than that found in the airlift fermenter, which was reported in Krusong *et al.* (2007); this was due to the lower DO, as mentioned earlier. The DO is the key parameter necessary for the oxidation reaction of *A. aceti* WK to convert alcohol to acetic acid. Consequently, the increase in DO in the STR is necessarily improved by using a proper impeller and aeration system.

During vinegar fermentation by *A. aceti* WK in the STR, a sample of loofa sponge was taken for scanning electron microscopy (x 2000). As shown in Figure 7, a large number of *A. aceti* WK cells ($3.43\text{--}5.21 \log \text{cfu/g}$) were supported on the surface of the loofa sponge. By observation, the number of cells varied directly with the surface area of the supporting sponge. This phenomenon

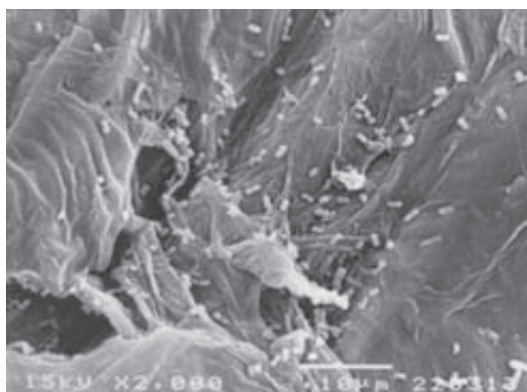


Figure 7 Scanning electron microscope (x 2000) showing cells of *A. aceti* WK on surface of loofa sponge during corn vinegar production in an STR.

could provide further information from previous study (Krusong *et al.*, 2007) that suggested if more cells of *A. aceti* WK were to remain in the reactor, more cycles of semi-continuous fermentation process for vinegar production could be conducted.

CONCLUSION

A cell recycle system was recommended for corn wine fermentation using *S. cerevisiae* M30, a flocculate yeast. Dried fibrous loofa sponge was confirmed as capable of supporting *A. aceti* WK cells, which adhered to the sponge surface and could be used for vinegar production in an STR. Using the semi-continuous process for corn vinegar production, a charging rate of 30% for filtered fresh corn wine was determined as the optimum for vinegar production in a 50-liter STR. An average acidity content of $4.1 \pm 0.1\%$ was observed during six cycles of fermentation within 40 d. Moreover, a shorter fermentation time was recorded after the first two cycles. This indicated that the *A. aceti* WK cells had successively adapted to the semi-continuous conditions in the STR.

Additionally, results confirmed that loofa sponge could be used for vinegar production in both an airlift fermenter and STR. Such a use supports the utilization of organic material for food as well as the green concept of being environment friendly, as the sponge can be easily and naturally decomposed and used as bio-fertilizer. Further study could investigate a suitable scale-up level using loofa material, as well as considering other organic fibrous materials.

Table 3 Acidification rate of *A. aceti* WK in an airlift fermenter and STR using loofa sponge for supporting material at 30°C.

Type of fermenter	Acidification rate (%/h)	Average acidification rate ² (%/h)
Airlift fermenter ¹	0.0031 – 0.0137	0.0084 ^a
STR	0.0028 – 0.0067	0.0047 ^b

¹ Result from Krusong *et al.* (2007)

² Mean followed by the same superscript in the same column indicates not significantly different by Duncan's new multiple range test ($P \leq 0.5$).

ACKNOWLEDGEMENTS

The authors would like to thank the Industrial Technology Assistance Program of the National Science and Technology Development Agency, Thailand, for supporting the research grant.

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